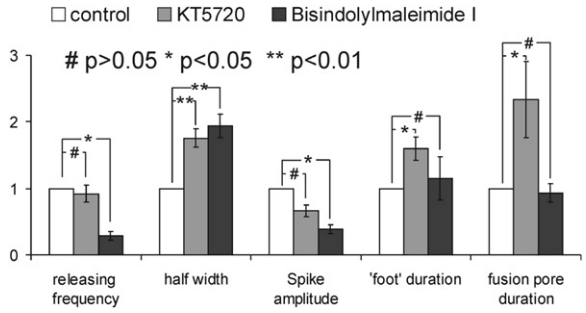
release, carbon fiber amperometry and cell-attached capacitance measurements were performed in bovine chromaffin cells. In cells treated with the PKC inhibitor, bisindolylmaleimide I (100nM, 10Ki), release frequency was significantly reduced to 5.0 ± 1.2events/min (from control 17.1 ± 2.0events/min). Amperometric spikes also showed increased half width of 22.3 ± 2.0ms (from 11.5 ± 0.9ms control) and smaller amperometric spike amplitude of 21.9 ± 3.7pA (from 56.2 ± 9.4pA control). In the cells treated with PKA inhibitor, KT 5720 (500nM, 10Ki), the duration of foot signals of amperometric events was prolonged to 14.4 ± 1.6ms (from 9.0 ± 1.0 ms control), consistent with prolonged fusion pore duration in cell-attached capacitance measurements of 27.7 ± 6.8ms (from 11.8 ± 1.4ms control). In contrast to PKC inhibitor, KT 5720 did not affect the release frequency.These results indicate that PKC affects the rate of fusion pore formation and release after full fusion but not early fusion pore expansion, while PKA specifically affects the expansion of the early fusion pore as well as release after full fusion.



524-Pos Board B403

Effects of Calcium and PIP2 on the Membrane Binding of Synaptotagmin I

Weiwei Kuo, Dawn Z. Herrick, David S. Cafiso.
University of Virginia, Charlottesville, VA, USA.

Synaptotagmin I (Syt I) appears to act as the Ca^{2+} sensor in neuronal exocytosis and it is known to interact both with membranes and with SNAREs, which form the conserved core protein machinery for the fusion process. The interactions of Syt I with membranes were examined here with a combination of vesicle sedimentation and site-directed spin labeling (SDSL). Several interesting features of the interaction are revealed. First, Syt I binds to PC/PS bilayers in a Ca^{2+}-independent manner though one of its cytosolic C2 domains, C2B. The interaction is mediated by the polybasic region of C2B domain, which associates in the electrostatic double-layer, but does not penetrate into the bilayer interior. Second, the affinity of C2B is increased approximately 20 fold in the presence of Ca^{2+} and now interacts through its Ca^{2+}-binding loops. Remarkably, in the presence of Ca^{2+}, C2A, C2B and a tandem fragment containing both C2A and C2B have approximately the same affinity, indicating the free energy of C2 domain interactions in Syt 1 are not additive. This may be due to demixing of the PS in the bilayer or the effects of curvature strain that are induced by the C2 domains. Finally, $PI(4,5)P_2$ is a lipid that is critical to membrane fusion. Our preliminary data indicate that the addition of 1 mol% $PI(4,5)P_2$ has little effect on the Ca^{2+}-dependent binding of C2A; however, the membrane binding of both C2B and the tandem C2A-C2B domains is enhanced by $PI(4,5)P_2$. As seen for other polybasic segments, the C2 domains appear to sequester or alter the lateral distribution of $PI(4,5)P_2$ in the bilayer.

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A Novel Approach For Wireless Communication Of *In Vivo* Data From Freely Moving Research Animals

Khajak Berberian[1], Alycia Gailey[1], Michael G. Kaplitt[2], Manfred Lindau[1].
[1]Cornell University, Ithaca, NY, USA, [2]Weill Medical College of Cornell University, New York, NY, USA.

In vivo electrochemistry has become a fascinating research tool allowing neuroscientists to study the release of oxidizable neurotransmitters, such as dopamine and norepinephrine in the brain of freely moving animals (Garris et al., 1997, J. Neurochem., 68(1): 152–161). The main limitation of this technique is the wired connection from the working electrode at the animal's head to the data acquisition apparatus, thus restricting the animal's freedom of motion. To overcome this limitation, we are designing an electronic device with the capability of performing fast-scan cyclic voltammetry measurements and wirelessly transmitting the recorded data. The device consists of two parts: the base station, which is connected to a PC, and the remote unit, which the rat carries on its back. The base station can wirelessly transmit the potential waveform applied to the working electrode, using the Advanced Audio Distribution Profile (A2DP) protocol. At the remote unit, a capacitance compensation circuit partially removes the capacitive background current present in voltammetric measurements due to charging of the Debye double layer. This increases the device's dynamic range, allowing for the detection of lower neurotransmitter levels. Although the forward telemetry (PC to remote unit) is functional, we have not yet characterized the reverse telemetry (remote unit to PC) in A2DP format. After finalizing the design, the device will be tested *in vivo* and subsequently employed in behavioral experiments, allowing researchers to obtain data from freely behaving rodents.

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Intracellular Ca^{2+} In Physiological Range Affects The Forward Rate Of Priming Of Large Dense Core Vesicles, But Not The Backward Rate

Mathias Pasche, Detlef Hof, Ulf Matti, Jens Rettig, Ute Becherer.
Saarland University, Homburg/Saar, Germany.

Secretory vesicles which undergo Ca^{2+}-dependent exocytosis pass several consecutive molecular states before release. While docking describes the anchoring of the vesicles to the plasma membrane, priming is necessary to render the vesicles release-competent. Many regulatory proteins and second messengers mediate the transition between these different molecular states.
By combining total internal reflection fluorescence microscopy (TIRFM) and analysis of the caging diameter (CD) we show that different molecular prefusion states of large dense core vesicles (LDCVs) can be distinguished by their different mobility (Nofal et al., J. Neurosci. 2007, 27:1386–95). Furthermore, we established simultaneous TIRFM measurements with whole-cell patch-clamp recordings which enables us to set a stable composition of the intracellular conditions, e.g. intracellular Ca^{2+} (Becherer et al., PLoS ONE 2007, 6:e505).
We investigated the Ca^{2+} dependence of both priming and unpriming reactions by varying the intracellular Ca^{2+} concentration within the physiological range from 50–800 nM.
CD analysis reveals that both lateral and axial mobility of LDCVs under resting conditions (100 nM $[Ca^{2+}]_i$) are elevated, whereas mobilities are reduced with raising $[Ca^{2+}]_i$ from 200 nM to 800 nM. Further increases of Ca^{2+} levels above 800 nM again lead to an increase in mobility. Interestingly, the dwell time of LDCVs appear to be independent of $[Ca^{2+}]_i$ in this range, arguing against the Ca^{2+}-dependence of docking. Quantitative analysis of individual parameters, such as dwelltime in a specific molecular state and frequency of interstate changes, demonstrate that the forward rate of priming is increased with raising $[Ca^{2+}]_i$ while the backward rate remains unaffected.

527-Pos Board B406

Role Of SPIN90 (SH3 Protein Interacting With Nck, 90kda) In The Formation Of Endocytic Vesicle And Its Movement In Receptor-mediated Endocytosis

Hye Jin Oh[1,2].
[1]Bio Imaging Research Center, GIST, Gwangju metropolitan city, Republic of Korea, [2]Cell Dynamics Research Center, GIST, Gwangju metropolitan city, Republic of Korea.

Endocytosis is a key mechanism for mediating diverse cellular functions, like uptake of nutrients, recycling of synaptic vesicles and intracellular signaling. The formation and targeting of vesicles to their acceptor compartment from the plasma membrane are tightly controlled for regulating tissue homeostasis. To gain insight into the effect of SPIN90 in the formation of vesicles, we measured the interaction between syndapin and dynamin in SPIN90 overexpressed and deficient fibroblasts. It is reported that syndapin is the phophorylation-regulated dynamin I partner in vivo and its interaction is crucial for SVE. SPIN90-SH3 domain binds with dynamin I-PRD in synapses and PRD domain of SPIN90 interacts to syndapin-SH3 in fibroblasts are already reported. Here, we show that the syndapin-dynamin interaction is maintained in SPIN90-N terminal (SH3 and PRD domain containing part) overexpressed cells comparing to that in mock overexpressed cells. In addition, SPIN90 C terminus (642–722aa) interacts with Rab5a small GTPase which has a role for early endosome movement and fusion were found. For verifying this, immuno-fluorescence and live cell imaging technique were used. We examined that SPIN90 is co-localized with Rab5 in fibroblast, and the movement of gfp-Rab5 positive endosome is delayed when the SPIN90-CC (Rab5 binding) part is overexpressed. From these results, we proposed that SPIN90 has a role in the formation and movement of early endosome.

528-Pos Board B407

TIRF-FRET As An Approach To Quantitative Analysis Of Dynamic Molecular Interactions On Secretory Granules In Live Cells

Alice D. Lam, Edward L. Stuenkel.
University of Michigan, Ann Arbor, MI, USA.

Secretory granules transit through a series of highly regulated associations and dissociations of molecular interactions enroute to membrane fusion and exocytotic release of the granule content. The ability to sequence these protein-protein interactions as they occur in living cells in real-time, with high spatial resolution, is paramount to furthering our understanding of how they relate to the functional state of the secretory granule as it transits the regulated exocytotic pathway. In the present study we have taken both an experimental and a theoretical approach to gain a quantitative understanding of the effects of evanescent illumination on sensitized-emission FRET calibrations and measurements, under a variety of conditions that mimic differing subcellular localizations of interacting molecules. Our results demonstrate that the TIRF-FRET method is straightforward for simple situations in which both donor and acceptor are on the same molecule and localized to the plasma membrane. By comparison when donor and acceptor molecules are localized to multiple intracellular compartments and where one compartment may be mobile, additional considerations must be taken into account. Our results define several of the parameters that are critical to the quantitative application of this method in living cells. Moreover, we demonstrate use of TIRF-FRET to visualize and quantify a specific set of bi-molecular interactions on insulin secretory granules in Min6 cells as they occur in time and subcellular space within the cell and we correlate these to the secretory event. This work supported by NIH, NINDS 039914 and NIDDK 053978.

529-Pos Board B408

Patterning Single Cell-Electrode Pairs for Electrochemical Measurement of Quantal Exocytosis on Microchips

Xin Liu, Syed Barizuddin, Cherian J. Mathai, Shubhra Gangopadhyay, Kevin D. Gillis.
University of Missouri-Columbia, Columbia, MO, USA.

We are developing transparent multi- electrochemical electrode arrays on microchips in order to automate measurement of quantal exocytosis. Design goals are that one and only one cell be positioned directly over each electrode and working electrodes have μm-scale dimensions in order to resolve pA-level currents. Patterning of cell-adhesion molecules in register with electrodes using conventional photolithographic approaches is problematic because organic solvents can disable sensitive biomolecule films. We report the parylene ''dry liftoff'' approach pioneered by Ilic and Craighead (Biomed Microdev 2: 317, 2000) can be used to pattern single cell-electrode pairs on the chip. A 1 μm-thick parylene C film is deposited on the multi-electrode array and S1813 photoresist is spin coated onto the device and patterned. The unprotected parylene over the electrodes is then removed using Reactive Ion Etch. Poly-l-lysine (PLL) is then added to promote cell attachment. Chromaffin cells are loaded on the chip in standard culture media and left in an incubator overnight. Finally, the parylene film is peeled off to remove excess cells and PLL, leaving tightly adhered chromaffin cells at the desired locations. Importantly, we find that promoting cell attachment with PLL films does not passivate the electrochemical electrodes. Experiments are in process to explore an alternative approach whereby PLL is patterned using the dry liftoff approach but cells are added after peel off of the parylene. With this approach, cell attachment to inactive areas of the chip is blocked by using ''cytophobic'' materials such as Teflon AF. This alternative approach may allow efficient targeting of cells at lower cell densities as cells migrate from cytophobic areas to the electrode binding sites (Supported by NIH BRP grant RO1 NS048826).

530-Pos Board B409

Endophilin N-BAR Domains-induced Membrane Remodeling Revealed by Molecular Dynamics Simulations

Haosheng Cui, Gary S. Ayton, Gregory A. Voth.
Center for Biophysical Modeling and Simulation, University of Utah, Salt Lake City, UT, USA.

Endophilin N-BAR domains play a critical rule in membrane remodeling (e.g., endocytosis, synapses) due to their membrane sculpting abilities. Presently, roles of the amphipathic helices and the positively charged concave surface on the crescent dimer in membrane remodeling are still not well understood. In addition, the endophilin N-BAR domain has one additional inserted helix on each of the monomers, thus making it unique in the entire BAR superfamily. Both the structure and the function of this additional helix are unknown up to now. Interestingly, the tubulated structures of endophilin N-BAR domains are much larger than the corresponding amphiphysin N-BAR domains. It is important to investigate the effect of the inserted helices in order to fully understand the mechanism of endophilin N-BAR domain protein driven liposome tubulation. Large scale all-atom molecular dynamics simulations are used to examine the details of the endophilin mediated membrane remodeling process. By comparing the results of different possible arrangements of the protein and membrane, we predict the optimum location of the additional helix. These results will facilitate in understanding the overall mechanism of endophilin N-BAR domains membrane oligomerization and remodeling.

531-Pos Board B410

Chronic Palmitate Exposure Inhibits Insulin Secretion By Dissociation of Ca^{2+}-Channels From Secretory Vesicles

Michael B. Hoppa[1], Stephan Collins[1], Reshma Ramracheya[1], Paul Johnson[2], Frances M. Ashcroft[1], Patrik Rorsman[1].
[1]Oxford University, Oxford, United Kingdom, [2]John Radcliffe Hospital, Headington, United Kingdom.

Type-2 diabetes, characterized by insufficient insulin secretion, shows a strong correlation with obesity and elevated plasma levels of free fatty acids (FFA). Long-term exposure of pancreatic islets to FFAs results in marked suppression of glucose-induced insulin secretion. Although the latter effect has been extensively characterized, the cellular mechanisms remain enigmatic. We have examined the effect of long-term exposure of pancreatic β-cells to palmitate using a combination of elecrophysiology and evanescent field microscopy. Here we show that rapid exocytosis in β-cells requires discrete microdomains of Ca^{2+}-entry close to the secretory vesicles and that this arrangement becomes disrupted following palmitate exposure. This culminates in the selective suppression of insulin release during brief (<50 ms) action potential-like stimulation whereas exocytosis evoked by unphysiologically long (>300 ms) pulses is unaffected. Additionally, inclusion of the slow Ca^{2+}-buffer EGTA (10 mM) in the electrode solution reversed the restored secretion observed during long pulses. Prolongation of the β-cell action potential by pharmacological maneuvers which expand the $[Ca^{2+}]_i$ microdomains corrects the FFA-induced secretion defect in both mouse and human islets. We propose that the FFA-induced dissociation of Ca^{2+}-entry from vesicles in β-cells selectively impairs the readily-releasable pool of vesicles but leaves vesicle docking with the membrane unaffected. This finding may represent an evolutionarily preserved mechanism to abate insulin secretion during nutrient deprivation when normoglycaemia is maintained by mobilization of lipids from fat depots.

Endoplasmic Reticulum & Protein Trafficking

532-Pos Board B411

Conformational transition of the Sec translocon induced by channel partner: A molecular dynamics study

Takaharu Mori[1,2], Ryuichiro Ishitani[3], Tomoya Tsukazaki[3], Osamu Nureki[3], Yuji Sugita[1,2].
[1]RIKEN, Saitama, Japan, [2]JST, BIRD, Saitama, Japan, [3]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Sec translocon is a highly conserved heteromeric membrane protein complex, which functions as a protein-conducting channel. In bacteria, the Sec translocon (SecYEG) achieves the translocation of polypeptides across the membrane by binding of the channel partner, SecA ATPase. However, little is known about the atomically detailed mechanism on the translocation. Recently, a new crystal structure of the SecYE translocon bound with an anti-SecY Fab fragment has been determined. It contains a large hydrophobic crevasse open to the cytoplasm (the pre-open form) and differ from the crystal structure of SecYEβ from *Methanococcus jannaschii* in the closed form, suggesting that the binding of a channel partner induces a large conformational change of the Sec translocon in the initial step of the polypeptide translocation. To investigate the role of channel-partner binding to the SecYE translocon, we performed all-atom molecular dynamics simulations of SecYE with and without a Fab fragment in explicit membrane. During a 100-ns simulation, SecYE undergoes a large conformational transition toward the closed form in the absence of a Fab fragment, whereas the structure keeps the widely opened crevasse in the simulation of SecYE with a Fab fragment. In the transition, protein-lipid interaction around the lateral gate region of SecYE is changed greatly, indicating that there is a competition of interactions between the protein and phospholipid molecules, which is controlled by the binding of the channel partner to the SecYE transolon.

533-Pos Board B412

Simulations of Multi-protein Complexes: Structure, Binding Affinity, and Dynamics of Vps27/hse1 Bound to Membrane-tethered Ubiquitin

Youngchan Kim, James H. Hurley, Gerhard Hummer.
National Institutes of Health, Bethesda, MD, USA.

Protein-protein interactions play an essential role in many cellular functions. While biophysical and structural characterizations have traditionally focused on strong binary complexes, the biological importance of weakly bound multi-protein complexes is increasingly recognized. Such complexes typically contain various proteins, with different folded domains held together in part by flexible linkers. Further increasing the complexity, many multi-protein